

Methods for Stabilizing Proteins

Claims

Claim 1. A process of stabilizing pyrene actin which requires concentrating the labeled protein to greater than 10mg/ml, preferably greater than 20mg/ml and most preferably greater than 30mg/ml prior to freezing and lyophilization.

Claim 2. A process of stabilizing pyrene-actin that incorporates the essence of Claim 1 and additionally requires rapid freezing prior to lyophilization, preferably in a dry ice ethanol bath, and most preferably in liquid nitrogen.

Claim 3. A process of stabilizing pyrene actin's activity to be more like the freshly prepared form, by including high amounts of a reducing agent prior to lyophilization.

Claim 4. A formula for pyrene actin lyophilization that allows storage of pyrene actin for greater than 3 years at 4°C, which is greater than 10mg/ml pyrene actin, preferably greater than 20mg/ml pyrene actin and most preferably greater than 30mg/ml pyrene actin, plus 5mM Tris-HCl pH8.0, 0.2mM ATP, 0.2mM CaCl₂, 5% w/v sucrose, 1% w/v dextran and 10mM DTT.

Claim 5. A method of reconstituting lyophilized pyrene actin so that it recreates the character of freshly prepared pyrene actin, which includes resuspending pyrene actin to 0.4mg/ml in A-buffer (5 mM Tris pH8, 0.2mM CaCl₂, 0.2mM ATP, also called G-buffer) and incubating for 1h on ice to depolymerize oligomers of actin that form during the preparation process, and preferably an

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additional 100,000 x g centrifugation for 2h to remove any remaining oligomers, prior to performing polymerization studies.